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DATE: Wednesday, November 26, 2003

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L1	map near2 kinase	1242	L1
L2	L1 same (dual or double or multiple or two or 2) same (phosphor$ or phosphat$)	444	L2
L3	L2 same (substrate or peptide or polypeptide or poly-peptide or protein or amino or epitope)	397	L3
L4	L3 same (method or assay or assaying or detect or determine or detecting or evaluating or identifying or identified or test or testing or screening or screen or evaluation)	179	L4
L5	l4 same (monoclonal or antibody or antibodies or antisera or antiserum or mono-clonal or moab or mab or scfv or antipeptide or antisubstrate)	31	L5

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☐ 1. Document ID: US 6649391 B1

L5: Entry 1 of 31 File: USPT Nov 18, 2003

DOCUMENT-IDENTIFIER: US 6649391 B1
TITLE: DSP-11 dual-specificity phosphatase

Detailed Description Text (37):
For example, [.sup.32 P]-radiolabeled substrate (e.g., MAP-kinase) may be used for the kinase reaction, resulting in radiolabeled, activated MAP-kinase. A DSP-11 polypeptide may then be tested for the ability to dephosphorylate an activated MAP-kinase by contacting the DSP-11 polypeptide with the MAP-kinase under suitable conditions (e.g., Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin for 10 minutes at 30.degree. C.; or as described by Zheng and Guan, J. Biol. Chem. 268:16116-16119, 1993). Dephosphorylation of the MAP-kinase may be detected using any of a variety of assays, such as a coupled kinase assay (evaluating phosphorylation of a MAP-kinase substrate using any assay generally known in the art) or directly, based on (1) the loss of radioactive phosphate groups (e.g., by gel electrophoresis, followed by autoradiography); (2) the shift in electrophoretic mobility following dephosphorylation; (3) the loss of reactivity with an antibody specific for phosphotyrosine or phosphothreonine; or (4) a phosphoamino acid analysis of the MAP-kinase. Certain assays may generally be performed as described by Ward et al., Nature 367:651-654, 1994 or Alessi et al., Oncogene 8:2015-2020, 1993. In general, contact of 500 pg-50 ng of DSP-11 polypeptide with 100 ng-100 .mu.g activated MAP-kinase should result in a detectable dephosphorylation of the MAP-kinase, typically within 20-30 minutes. Within certain embodiments, 0.01-10 units/mL (preferably about 0.1 units/mL, where a unit is an amount sufficient to dephosphorylate 1 nmol substrate per minute) DSP-11 polypeptide may be contacted with 0.1-10 .mu.M (preferably about 1 .mu.M) activated MAP-kinase to produce a detectable dephosphorylation of a MAP-kinase. Preferably, a DSP-11 polypeptide results in a dephosphorylation of a MAP-kinase or a phosphorylated substrate (such as a tyrosine-and/or serine-phosphorylated peptide) that is at least as great as the dephosphorylation observed in the presence of a comparable amount of native human DSP-11. It will be apparent that other substrates identified using a substrate trapping mutant as described herein may be substituted for the MAP-kinase within such assays.

Detailed Description Text (78):
In one aspect of the present invention, DSP-11 polypeptides may be used to identify agents that modulate DSP-11 activity. Such agents may inhibit or enhance signal transduction via a MAP-kinase cascade, leading to cell proliferation. An agent that modulates DSP-11 activity may alter expression and/or stability of DSP-11 , DSP-11 protein activity and/or the ability of DSP-11 to dephosphorylate a substrate. Agents that may be screened within such assays include, but are not limited to, antibodies and antigen-binding fragments thereof,

competing substrates or peptides that represent, for example, a catalytic site or a dual phosphorylation motif, antisense polynucleotides and ribozymes that interfere with transcription and/or translation of DSP-11 and other natural and synthetic molecules, for example small molecule inhibitors, that bind to and inactivate DSP-11.

☐ 2. Document ID: US 6645753 B1

L5: Entry 2 of 31 File: USPT Nov 11, 2003

DOCUMENT-IDENTIFIER: US 6645753 B1
TITLE: DSP-5 dual-specificity phosphatase

Detailed Description Text (39):
For example, [.sup.32 P]-radiolabeled substrate (e.g., MAP-kinase) may be used for the kinase reaction, resulting in radiolabeled, activated MAP-kinase. A DSP-5 (or DSP-5 alternate form) polypeptide may then be tested for the ability to dephosphorylate an activated MAP-kinase by contacting the DSP-5 polypeptide with the MAP-kinase under suitable conditions (e.g., Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin for 10 minutes at 30.degree. C.; or as described by Zheng and Guan, J. Biol. Chem. 268:16116-16119, 1993). Dephosphorylation of the MAP-kinase may be detected using any of a variety of assays, such as a coupled kinase assay (evaluating phosphorylation of a MAP-kinase substrate using any assay generally known in the art) or directly, based on (1) the loss of radioactive phosphate groups (e.g., by gel electrophoresis, followed by autoradiography); (2) the shift in electrophoretic mobility following dephosphorylation; (3) the loss of reactivity with an antibody specific for phosphotyrosine or phosphothreonine; or (4) a phosphoamino acid analysis of the MAP-kinase. Certain assays may generally be performed as described by Ward et al., Nature 367:651-654, 1994 or Alessi et al., Oncogene 8:2015-2020, 1993. In general, contact of 500 pg--50 ng of DSP-5 polypeptide with 100 ng-100 .mu.g activated MAP-kinase should result in a detectable dephosphorylation of the MAP-kinase, typically within 20-30 minutes. Within certain embodiments, 0.01-10 units/mL (preferably about 0.1 units/mL, where a unit is an amount sufficient to dephosphorylate 1 nmol substrate per minute) DSP-5 polypeptide may be contacted with 0.1-10 .mu.M (preferably about 1 .mu.M) activated MAP-kinase to produce a detectable dephosphorylation of a MAP-kinase. Preferably, a DSP-5 polypeptide results in a dephosphorylation of a MAP-kinase or a phosphorylated substrate (such as a tyrosine- and/or serine-phosphorylated peptide) that is at least as great as the dephosphorylation observed in the presence of a comparable amount of native human DSP-5 (or DSP-5 alternate form). It will be apparent that other substrates identified using a substrate trapping mutant as described herein may be substituted for the MAP-kinase within such assays.

Detailed Description Text (81):
In one aspect of the present invention, DSP-5 (or DSP-5 alternate form) polypeptides may be used to identify agents that modulate DSP-5 activity. Such agents may inhibit or enhance signal transduction via a MAP-kinase cascade, leading to cell proliferation. An agent that modulates DSP-5 (or DSP-5 alternate form) activity may alter expression and/or stability of DSP-5, DSP-5 protein activity and/or the ability of DSP-5 to

dephosphorylate a substrate. Agents that may be screened within such assays include, but are not limited to, antibodies and antigen-binding fragments thereof, competing substrates or peptides that represent, for example, a catalytic site or a dual phosphorylation motif, antisense polynucleotides and ribozymes that interfere with transcription and/or translation of DSP-5 (or DSP-5 alternate form) and other natural and synthetic molecules, for example small molecule inhibitors, that bind to and inactivate DSP-5 (or DSP-5 alternate form).

☐ 3. Document ID: US 6632628 B1

L5: Entry 3 of 31 File: USPT Oct 14, 2003

DOCUMENT-IDENTIFIER: US 6632628 B1
TITLE: Methods and compositions relating to HDAC 4 and 5 regulation of cardiac gene expression

Detailed Description Text (270):
The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference. U.S. Pat. No. 5,359,046 U.S. Pat. No. 4,367,110 U.S. Pat. No. 4,452,901 U.S. Pat. No. 4,668,621 U.S. Pat. No. 4,873,191 U.S. Pat. No. 5 ,708,158 U.S. Pat. No. 5,252,479 U.S. Pat. No. 5,672,344 WO 84/03564 Adolph et al., "Role of myocyte-specific enhancer-binder factor (MEF-2) in transcriptional regulation of the acardiac myosin heavy chain gene," J. Biol. Chem., 268:5349-5352, 1993. Baichwal and Sugden, In: Gene Transfer, Kucherlapati R, ed., New York, Plenum Press, 117-148, 1986. Batterson and Roizman, J. Virol., 46:371-377, 1983. Bedzyk et al., J. Biol. Chem., 265:18615, 1990. Bellon et al., de Ses Filiales, 190(1):109-142, 1996. Benvenisty and Neshif, Proc. Nat'l Acad. Sci. USA, 83:9551-9555, 1986. Berns and Bohenzky, Adv. Virus Res., 32:243-307, 1987. Berns and Giraud, Curr. Top. Microbiol. Immunol., 218:1-23, 1996. Berns, Microbiol Rev., 54:316-329, 1990. Bertran et al., J. Virol., 70(10):6759-6766, 1996. Bito et al., "CREB Phosphorylation and Dephosphorylation: Aca2+- and Stimulus Duration-Dependent Switch for Hippocampal Gene Expression," Cell,. 87:1203-1214, 1996. Botinelli et a., Circ. Res. 82:106-115, 1997. Bour et al., "Drosophila MEF2, a transcription factor that is essential for myogenesis," Genes and Dev., 9:730-741, 1995. Bowman et al., "Expression of Protein Kinase C B in the Heart Causes Hypertrophy in Adult Mice and Sudden Death in Neonates," J. Clin. Invest., 100:2189-2195, 1997. Brand, "Myocyte enhancer factor 2 (MEF2)," Int J. Biochem. Cell Biol., 29:1467-1470; 1997. Brinster et al., Proc. Nat'l Acad. Sci. USA, 82: 4438-4442, 1985. Brown et al., J. Neurochem. 40:299-308, 1983. Bustamante et al., J. Cardiovasc. Pharmacol, 17: S110-113, 1991. Chaudhary et al., Proc. Nat'l Acad. Sci., 87:9491, 1990. Chen and Okayama, Mol. Cell Biol., 7:2745-2752, 1987. Chien et al., Ann. Rev. Physiol. 55, 77-95, 1993. Chien et al., "Regulation of cardiac gene expression during myocardial growth and hypertrophy: Molecular studies of an adaptive physiologic response," FASEB J., 5:3037-3046, 1991. Chomczynski and Sacchi, Anal. Biochem., 162:156-159, 1987. Clarke et al., "Epidermal Growth Factor Induction of the c-jun Promoter by a Rac Pathway," Mol. Cell Biol., 18:1065-1073, 1998. Coffin, In., Fields BN, Knipe DM, ed. VIROLOGY. New York: Raven Press, pp. 1437-1500, 1990. Colbert et al., "Cardiac Compartment-specific Overexpression of a Modified Retinoic Acid Receptor Produces Dilated Cardiomyopathy and Congestive Heart Failure in Transgenic Mice," J. Clin. Invest., 100: 1958-1968, 1997. Coso et al., "Signaling from G Protein-coupled Receptors to the c-jun promoter

Involves the MEF2 Transcription Factor," J. Biol. Chem., 272:20691-20697, 1997. Couch et al., Am. Rev. Resp. Dis., 88:394-403, 1963. DeLuca et al., J. Virol., 56:558-570, 1985. Dolmetsch et al., "Differential activation of transcription factors induced by Ca.sup.2+ response amplitude and duration," Nature, 386:855-858, 1997. Dubensky et al, Proc. Nat'l Acad. Sci. USA, 81:7529-7533, 1984. Edmondson et al., "MEF2 gene expression marks the cardiac and skeletal muscle lineages during mouse embryogenesis," Development, 120:1251-1263, 1994. Ellis et al., "Replacement of insulin receptor tyrosine residues 1162 and 1163 compromises insulin-stimulated kinase activity," 1986. Elroy-Stein et al., Proc. Nat'l Acad. Sci. USA, 1989. Elshami et al., Gene Therapy, 7(2):141-148, 1996. Emmel et al., "Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation," Science, 246:1617-1620, 1989. 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Mulligan, Science, 260:926-932, 1993. Myers, EPO 0273085. Nicolas and Rubenstein, In: Vectors: A survey of molecular cloning vectors and their uses. Rodriguez and Denhardt (eds.), Stoneham: Butterworth, pp. 494-513, 1988. Nicolau and Sene, Biochem.

☐ 4. Document ID: US 6613956 B1

L5: Entry 4 of 31 File: USPT Sep 2, 2003

DOCUMENT-IDENTIFIER: US 6613956 B1
TITLE: PI 3-kinase fusion mutants and uses thereof

Detailed Description Text (106):
Cell lysates containing HA-tagged pp70 S6 kinase, Akt-kinase, MAP-kinase or cJun terminal kinase (JNK) were incubated with monoclonal anti-HA antibody 12CA5 for 1 hour at 4.degree. C. Protein A-Sepharose beads (Sigma, St. Louis, Mo.) were used to precipitate the immune complexes. The beads were washed with 50 mM Tris-HCl (pH 7.5), 0.5 M LiCl, 0.5% v/v Triton X-100, twice with PBS and once with 10 mM Tris-HCl (pH 7.5), 10 mM MgCl.sub.2, 1 mM dithiothreitol, all containing 0.1 mM sodium vanadate and 20 mM .beta.-glycerolphosphate. For analyzing the immune complexes in an S6 kinase activity assay the beads were divided in three aliquots: two aliquots were subjected to a S6 kinase activity assay using [.lambda.-.sup.32 p] ATP (5,000 Ci/mmol) based on a peptide substrate described in Terada et al, J. Biol. Chem. 268: 12062-8 (1993), in 30 .mu.l, one aliquot was analyzed for the amount of recombinant pp70 S6 kinase in the precipitate. After 25 minutes at 22.degree. C. the reaction was stopped by the addition of 10 .mu.l of 500 mM EDTA. Twenty two .mu.l of the supernatant was applied to phosphocellulose paper P81 made by Whatman Products, Fisher Scientific, Pittsburg, Pa., and washed four times in 75 mM H.sub.3 PO.sub.4. The relative amounts of incorporated radioactivity was determined in a liquid scintillation counter. Specific phosphorylation of the S6-derived peptide was obtained after subtracting counts with protein A-Sepharose beads in the absence of anti-HA-antibody from counts of label incorporated in the presence of anti-HA-antibody.

Detailed Description Text (107):
For all the other kinase assays, one-third of the immunobeads were subjected to an in vitro kinase reaction, and two-thirds were analyzed for the amount of the respective recombinant kinase protein. For analyzing Akt kinase activity histone H2B was used as substrate as described in Franke et al., Cell 81:727-36 (1995), according to the reaction conditions described by Jones et al, PNAS USA 88:4171-5 (1991). JNK-activity was determined using GST-Jun (amino acids of Jun 1 through 89, which a slight variation from a standard version that contains amino acids 1 through 79) as a substrate as described in Derijard et al., Cell 76:1025-37 (1994). For MAP kinase activation, the phosphorylation of myelin basic protein (MBP) was analyzed as described by Ray et al., PNAS USA 85:3753-7 (1988). The in vitro protein kinase reactions were carried out in 30 .mu.l in the presence of [y-.sup.32 p] ATP (5,000 Ci/mmol) and incubated at 22.degree. C. for 25 minutes. The reactions were stopped by the addition of 8 .mu.l Lammli-sample buffer and 22 .mu.l of the reaction mixtures were analyzed by SDS-PAGE. The relative amounts of incorporated radioactivity was determined by autoradiography and quantitated using a Molecular Imager System produced by BioRad, Richmond, Calif. The complexes were analyzed by immunoblotting with the indicated antibodies.

☐ 5. Document ID: US 6610523 B1

L5: Entry 5 of 31 File: USPT Aug 26, 2003

DOCUMENT-IDENTIFIER: US 6610523 B1
TITLE: Cytokine-, stress-, and oncoprotein-activated human protein kinase kinases

Detailed Description Text (144):
To examine the specificity of MKK7 in vivo, cotransfection assays were performed. CHO cells were maintained in Dulbecco's modified Eagle's medium supplemented with fetal calf serum (5%; Gibco-BRL). The cells were transfected with the lipofectamine reagent according to the manufacturer's recommendations (Gibco-BRL)(Derijard (1994) supra). Cells were co-transfected with vectors encoding epitope-tagged JNK1 together with an empty expression vector (control) or an expression vector encoding MKK4 or MKK7. The epitope tag was derived from the hemagglutinin protein (HA) of the influenza virus. JNK1 was isolated by immunoprecipitation of cell lysates. The cells were solubilized with lysis buffer (20 mM Tris (pH 7.4), 1% TRITON X-100.RTM., 10% glycerol, 137 mM NaCl, 2 mM EDTA, 25 mM .beta.-glycerophosphate, 1 mM Na orthovanadate, 2 mM pyrophosphate, 1 mM PMSF, 10 .mu.g/ml leupeptin) and centrifuged at 100,000.times.g for 15 minutes at 4.degree. C. The epitope-tagged protein kinases were immunoprecipitated by incubation for 3 hours at 4.degree. C. with an anti-HA monoclonal antibody bound to protein-G Sepharose (Pharmacia-LKB Biotechnology Inc.). The immunoprecipitates were washed three times with lysis buffer (Gupta et al. (1995) Science 267:389-393). Protein kinase activity was measured in the immunecomplex with [.gamma.-.sup.32 P]ATP and c-Jun as substrates. The product of the phosphorylation reaction was visualized after SDS-PAGE by autoradiography. The ERK2 and p38 MAP kinases were not activated by co-expressed MKK7. Control experiments demonstrated that the ERK2 and p38 MAP kinases were activated by their respective cognate MAP kinase kinases, MKK1 and MKK6. In contrast, MKK7 did activate JNK1. Interestingly, the activation of JNK1 by co-expressed MKK7 was greater than that caused by the previously described JNK activator MKK4. Together, these data establish that MKK7 can function as a specific activator of JNK in cultured cells.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

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☐ 6. Document ID: US 6586191 B2

L5: Entry 6 of 31 File: USPT Jul 1, 2003

DOCUMENT-IDENTIFIER: US 6586191 B2
TITLE: Method of identifying compounds that bind galanin receptor (GALR2)

Detailed Description Text (233):
CHO cells expressing either the rat GALR2 receptor or the human GALR2 receptor were plated at .about.50%

confluence into well-plates and grown in culture with fetal bovine serum reduced to 0.5% for 2 days. Fresh medium was exchanged for old and then aspirated after 2 hrs. The assay was initiated by adding fresh medium plus or minus 1 uM human galanin for 10 minutes. Cells were washed with phosphate buffered saline and lysed in 100 uL Laemmli sample buffer (Bio-Rad) containing 5% .beta.-mercaptoethanol. Cell lysate was scraped off the plate, transferred to a microfuge tube on ice, and sonicated for 10-15 seconds. Samples were heated at 100.degree. C. for 3-5 minutes. Insoluble material was collected by centrifugation at 10,000.times.g for 5 min. Supernatant (20 .mu.L/lane) was loaded onto a 4-20% polyacrylamide Bio-Rad Ready-Gel (10.times.10 cm) together with a phospho-MAP kinase protein standard (New England BioLabs) and molecular weight markers. Proteins were fractionated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred by electrophoresis to a PVDF membrane (Bio-Rad). The membrane was blocked with 5% nonfat dry milk. Phosphorylated MAP kinase was labeled with a rabbit anti-phospho-MAP kinase antibody (New England New England BioLabs) and HRP-conjugated goat anti-rabbit IgG according to standard western blot procedure. The phospho-MAP kinase complex was detected by chemiluminescence using Lumiglow reagent (New England BioLabs) and Kodak X-Omat film, with exposure times ranging from 10 to 120 seconds.

☐ 7. Document ID: US 6579691 B1

L5: Entry 7 of 31 File: USPT Jun 17, 2003

DOCUMENT-IDENTIFIER: US 6579691 B1
TITLE: Protein kinase NPK-110

Detailed Description Text (53):
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Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

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☐ 8. Document ID: US 6576437 B2

L5: Entry 8 of 31 File: USPT Jun 10, 2003

DOCUMENT-IDENTIFIER: US 6576437 B2
TITLE: Stimulus-inducible protein kinase complex and methods of use therefor

Detailed Description Text (55):
NF.kappa.B activation is known to occur under conditions that also stimulate MAP kinase pathways (Lee et al., Cell 88:213-22, 1997; Hirano, et al., J. Biol. Chem. 271:13234-38, 1996). Accordingly, further experiments were performed to detect proteins associated with MAP kinase and phosphatase cascades at various stages of purification of the IKK signalsome. In addition to RelA and I.kappa.B.beta., MEKK-1 and two tyrosine-phosphorylated proteins of .about.55 and .about.40 kDa copurified with I.kappa.B kinase activity (FIG. 1C). Antibodies to Rel A and I.kappa.B.beta. were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.), and antibodies to MEKK-1 were obtained from Upstate Biotechnology (Lake Placid, N.Y.). Other signaling components, including PKC.zeta., PP1 and PP2A, were detected in the same fractions as the I.kappa.B kinase in early chromatographic steps but did not copurify at later chromatographic steps (data not shown). Most interestingly, an unidentified protein of .about.50 kDa, detected by its crossreaction with an antibody to MKP-1, copurified with I.kappa.B kinase through several purification steps (FIG. 1C). This protein is unlikely to be MKP-1 itself, since the molecular weight of authentic MKP-1 is 38 kDa.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 9. Document ID: US 6551810 B1

L5: Entry 9 of 31 File: USPT Apr 22, 2003

DOCUMENT-IDENTIFIER: US 6551810 B1
TITLE: DSP-10 dual-specificity phosphatase

Detailed Description Text (37):
For example, [.sup.32 P]-radiolabeled substrate (e.g., MAP-kinase) may be used for the kinase reaction, resulting in radiolabeled, activated MAP-kinase. A DSP-10 polypeptide may then be tested for the ability to dephosphorylate an activated MAP-kinase by contacting the DSP-10 polypeptide with the MAP-kinase under suitable conditions (e.g., Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin for 10 minutes at 30.degree. C.; or as described by Zheng and Guan, J. Biol. Chem. 268:16116-16119, 1993). Dephosphorylation of the MAP-kinase may be detected using any of a variety of assays, such as a coupled kinase assay (evaluating phosphorylation of a MAP-kinase substrate using any assay generally known in the art) or directly, based on (1) the loss of radioactive phosphate groups (e.g., by gel electrophoresis, followed by autoradiography); (2) the shift in electrophoretic mobility following dephosphorylation; (3) the loss of reactivity with an antibody specific for phosphotyrosine or phosphothreonine; or (4) a phosphoamino acid analysis of the MAP-kinase. Certain assays may generally be performed as described by Ward et al., Nature

367:651-654, 1994 or Alessi et al., Oncogene 8:2015-2020, 1993. In general, contact of 500 pg-50 ng of DSP-10 polypeptide with 100 ng-100 .mu.g activated MAP-kinase should result in a detectable dephosphorylation of the MAP-kinase, typically within 20-30 minutes. Within certain embodiments, 0.01-10 units/mL (preferably about 0.1 units/mL, where a unit is an amount sufficient to dephosphorylate 1 nmol substrate per minute) DSP-10 polypeptide may be contacted with 0.1-10 .mu.M (preferably about 1 .mu.M) activated MAP-kinase to produce a detectable dephosphorylation of a MAP-kinase. Preferably, a DSP-10 polypeptide results in a dephosphorylation of a MAP-kinase or a phosphorylated substrate (such as a tyrosine- and/or serine-phosphorylated peptide) that is at least as great as the dephosphorylation observed in the presence of a comparable amount of native human DSP-10. It will be apparent that other substrates identified using a substrate trapping mutant as described herein may be substituted for the MAP-kinase within such assays.

Detailed Description Text (78):
In one aspect of the present invention, DSP-10 polypeptides may be used to identify agents that modulate DSP-10 activity. Such agents may inhibit or enhance signal transduction via a MAP-kinase cascade, leading to cell proliferation. An agent that modulates DSP-10 activity may alter expression and/or stability of DSP-10, DSP-10 protein activity and/or the ability of DSP-10 to dephosphorylate a substrate. Agents that may be screened within such assays include, but are not limited to, antibodies and antigen-binding fragments thereof, competing substrates or peptides that represent, for example, a catalytic site or a dual phosphorylation motif, antisense polynucleotides and ribozymes that interfere with transcription and/or translation of DSP-10 and other natural and synthetic molecules, for example small molecule inhibitors, that bind to and inactivate DSP-10.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

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☐ 10. Document ID: US 6511800 B1

L5: Entry 10 of 31 File: USPT Jan 28, 2003

DOCUMENT-IDENTIFIER: US 6511800 B1
**** See image for Certificate of Correction ****
TITLE: Methods of treating nitric oxide and cytokine mediated disorders

Detailed Description Text (403):
The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference. Abbas, A. K., et al., Cellular and Molecular Immunology, W. B. Saunders Co., Publisher, Philadelphia, Pa., 1991. Abbondanzo et al., Breast Cancer Res. Treat., 16:182(#151), 1990. Akamatsu, Ohno, Hirota, Kegoshima, Yodoi, Shigesada, "Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues," J. Biol. Chem. 272:14497-14500, 1997. Allred et al., Breast Cancer Res. Treat., 16:182(#149), 1990. Aruoma, O. I., et al., "The antioxidant action of N-acetyl cysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid," Free Rad. Biol. Med., 6:593-597, 1989. Bagasra, Michaels, Zheng, Bobroski, Spitsin, Fu, Tawadros, Koprowski, "Activation of the inducible form of nitric oxide

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☐ 11. Document ID: US 6500942 B1

L5: Entry 11 of 31 File: USPT Dec 31, 2002

DOCUMENT-IDENTIFIER: US 6500942 B1

**** See image for Certificate of Correction ****

TITLE: Rin2, a novel inhibitor of Ras-mediated signaling

Detailed Description Text (75):
The activation of p38 MAP kinase activity was assayed using the p38 MAP Kinase Assay Kit (New England BioLabs). Specifically, total p38 MAP kinase was immunoprecipitated using a p38 MAP kinase antibody and incubated with GST-ATF-2 fusion protein in the presence of ATP and kinase buffer. Phosphorylation of ATF-2 was measured by Western blotting using a phospho-specific ATF-2 antibody and ECL.

☐ 12. Document ID: US 6500674 B1

L5: Entry 12 of 31 File: USPT Dec 31, 2002

DOCUMENT-IDENTIFIER: US 6500674 B1
TITLE: Method for the diagnosis of brain/neurological disease using monoclonal antibodies specific for PHF-tau, hybridomas secreting them, and antigen recognition by these antibodies and their applications

Detailed Description Text (41):
REFERENCES Andreadis A, Brown W, Kosik K (1992) Structure and novel exons of the human tau gene. Biochem 31:10626-10633. Bramblett G, Trojanowski J, Lee V (1992) Regions with abundant neurofibrillary pathology in human brain exhibit a selective reduction in levels of binding-competent tau and accumulation of abnormal tau isoforms (A68 proteins). Lab Invest 66:212-222. Bramblett G, Goedert M, Jakes R, Merrick S, Trojanowski J, Lee V (1993) The abnormal phosphorylation of tau at Ser396 in Alzheimer's disease recapitulates phosphorylation during development and contributes to reduced microtubule binding. Neuron 10:1089-1099. Brion J, Couck A, Passareiro E, Flament-Durand J (1985a) Neurofibrillary tangles of Alzheimer's disease: an immunohistochemical study. J Submicrosc Cytol 17:89-96. Brion J, Passareiro J, Nunez J and Flament-Durand J (1985) Mise en evidence immunologique de la proteine tau au niveau des lesions de degenerescence neurofibrillaire de la maladie d'Alzheimer. Arch Biol 95:229-235. Biernat J, Mandelkow M, Schoter C, Lichtenberg-Kraag B, Steiner B, Berling B, Meyer H, Mercken M, Vandermeeren M, Mandelkow E, The switch of tau protein to an Alzheimer-like state includes the phosphorylation of two serine-proline motifs upstream of the microtubule binding region. EMBO J, 1992, 11:1593-1597. Bobrow M, Harris T, Shaughnessy K, Litt G (1989) Catalyzed reporter deposition, a novel method of signal amplification. Application to immunoassays. J Immunol Meth 125:279-285. Butner K, Kirschner (1991) Tau protein binds to microtubules through a flexible array of distributed weak sites. J Cell Biol 115:717-730. de Bont H, van Boom J, Liskamp R (1990a) Automatic synthesis of phosphopeptides by phosphorylation on the solid phase. Tetrahedron Letters 31:2497-2500. de Bont H, van Boom J, Liskamp R (1990b) N,N-diiopropyl-bis(4-chlorobenzyl)phosphoramidite: A versatile phosphitylating agent for the phosphorylation of hydroxy amino acids and preparation of protected phosphopeptides. Recueil des Travaux Chimiques des Pays-bas 109:27-28. Delacourte A, Flament S, Dibe E, Hublau P, Sablonniere B, Hemon B, Sherrer V, Defossez A (1990) Pathological proteins Tau64 and 69 are specifically expressed in the somatodendritic domain of the degenerating cortical neurons during Alzheimer's disease. Acta Neuropathol 80: 111-117. Delacourte A, Defossez A (1986) Alzheimer's disease: Tau proteins,

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Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 13. Document ID: US 6492363 B2

L5: Entry 13 of 31 File: USPT Dec 10, 2002

DOCUMENT-IDENTIFIER: US 6492363 B2
TITLE: 2-(4-bromo or 4-iodo phenylamino) benzoic acid derivatives

Detailed Description Text (77):
To determine the state of tyrosine phosphorylation of cellular MAP kinase, cells were lysed, endogenous MAP kinase was immunoprecipitated with a specific antibody, and the resulting immunoprecipitate analyzed for the presence of phosphotyrosine as follows: confluent cells were serum-deprived overnight and treated with compounds and growth factors as described above. Cells were then scraped and pelleted at 13,000.times.g for 2 minutes. The resulting cell pellet was resuspended and dissolved in 100 .mu.L of 1% SDS containing 1 mM NaVO.sub.4. Following alternate boiling and vortexing to denature cellular protein, 900 .mu.L RIPA buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% deoxycholate, and 10 mM EDTA) was added. To this mixture was added 60 .mu.L agarose beads coupled with rabbit immunoglobulin G and 60 .mu.L Pansorbin cells in order to clear the lysate of nonspecific binding proteins. This mixture was incubated at 4.degree. C. for 15 minutes then centrifuged at 13,000.times.g for 10 minutes. The resulting supernatant was transferred to fresh tubes and incubated with 10 .mu.L of a polyclonal antisera raised against a fragment of MAP kinase for a minimum of 1 hour at 4.degree. C. Seventy microliters of a slurry of agarose beads coupled with protein G and protein A was added and the incubation continued for an additional 30 minutes at 4.degree. C. The beads were pelleted by centrifugation at 13,000.times.g for 5 minutes and washed three times with 1 mL RIPA buffer. Laemmli sample buffer was added to the final bead pellet. This mixture was boiled for 5 minutes then resolved on a 10% acrylamide gel. Proteins on the gel were transferred to a nitrocellulose membrane and nonspecific binding sites on the membrane blocked by incubation with 1% ovalbumin and 1% bovine serum albumin in TBST (150 mM NaCl, 10 mM Tris (pH 7.4), and 0.05% Tween 20). The membrane was then incubated with a commercially available antibody directed against phosphotyrosine. Antibody bound on the membrane was detected by incubation with .sup.125 I-protein A, followed by autoradiography.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 14. Document ID: US 6492157 B1

L5: Entry 14 of 31 File: USPT Dec 10, 2002

DOCUMENT-IDENTIFIER: US 6492157 B1

TITLE: DSP-9 dual-specificity phosphatase

Detailed Description Text (37):
For example, [.sup.32 P]-radiolabeled substrate (e.g., MAP-kinase) may be used for the kinase reaction, resulting in radiolabeled, activated MAP-kinase. A DSP-9 polypeptide may then be tested for the ability to dephosphorylate an activated MAP-kinase by contacting the DSP-9 polypeptide with the MAP-kinase under suitable conditions (e.g., Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin for 10 minutes at 30.degree. C.; or as described by Zheng and Guan, J. Biol. Chem. 268:16116-16119, 1993). Dephosphorylation of the MAP-kinase may be detected using any of a variety of assays, such as a coupled kinase assay (evaluating phosphorylation of a MAP-kinase substrate using any assay generally known in the art) or directly, based on (1) the loss of radioactive phosphate groups (e.g., by gel electrophoresis, followed by autoradiography); (2) the shift in electrophoretic mobility following dephosphorylation; (3) the loss of reactivity with an antibody specific for phosphotyrosine or phosphothreonine; or (4) a phosphoamino acid analysis of the MAP-kinase. Certain assays may generally be performed as described by Ward et al., Nature 367:651-654, 1994 or Alessi et al., Oncogene 8:2015-2020, 1993. In general, contact of 500 pg-50 ng of DSP-9 polypeptide with 100 ng-100 .mu.g activated MAP-kinase should result in a detectable dephosphorylation of the MAP-kinase, typically within 20-30 minutes. Within certain embodiments, 0.01-10 units/mL (preferably about 0.1 units/mL, where a unit is an amount sufficient to dephosphorylate 1 nmol substrate per minute) DSP-9 polypeptide may be contacted with 0.1-10 .mu.M (preferably about 1 .mu.M) activated MAP-kinase to produce a detectable dephosphorylation of a MAP-kinase. Preferably, a DSP-9 polypeptide results in a dephosphorylation of a MAP-kinase or a phosphorylated substrate (such as a tyrosine- and/or serine-phosphorylated peptide) that is at least as great as the dephosphorylation observed in the presence of a comparable amount of native human DSP-9. It will be apparent that other substrates identified using a substrate trapping mutant as described herein may be substituted for the MAP-kinase within such assays.

Detailed Description Text (78):
In one aspect of the present invention, DSP-9 polypeptides may be used to identify agents that modulate DSP-9 activity. Such agents may inhibit or enhance signal transduction via a MAP-kinase cascade, leading to cell proliferation. An agent that modulates DSP-9 activity may alter expression and/or stability of DSP-9, DSP-9 protein activity and/or the ability of DSP-9 to dephosphorylate a substrate. Agents that may be screened within such assays include, but are not limited to, antibodies and antigen-binding fragments thereof, competing substrates or peptides that represent, for example, a catalytic site or a dual phosphorylation motif, antisense polynucleotides and ribozymes that interfere with transcription and/or translation of DSP-9 and other natural and synthetic molecules, for example small molecule inhibitors, that bind to and inactivate DSP-9.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

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☐ 15. Document ID: US 6486301 B1

L5: Entry 15 of 31 File: USPT Nov 26, 2002

DOCUMENT-IDENTIFIER: US 6486301 B1

TITLE: Interleukin-20

Detailed Description Text (395):
A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6 ng/well) or 50 ul of the supernatants obtained in Example 12 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate. After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10 ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1 ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by IL-20 or a molecule induced by IL-20.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 16. Document ID: US 6399633 B1

L5: Entry 16 of 31 File: USPT Jun 4, 2002

DOCUMENT-IDENTIFIER: US 6399633 B1
TITLE: Use of 4-H-1-benzopryan-4-one derivatives as inhibitors of smooth muscle cell proliferation

Drawing Description Text (6):
FIG. 5. Effects of flavopiridol on MAP kinase activity in HASMC. Quiescent HASMC were treated in the presence (+) or absence (-) of bFGF (10 ng/ml), thrombin (2 U/ml), PD98059 (30 .mu.mol/L) and/or flavopiridol (75 nmol/L) for 30 min. Levels of phosphorylated Erk1 (pErk1) and Erk2 (pErk2) were measured by immunoblotting with a phosphorylation-specific antibody recognizing both proteins (upper panel). MAP kinase activity was measured with an in-gel kinase assay, using myelin basic protein as a substrate (lower panel).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 17. Document ID: US 6350856 B1

L5: Entry 17 of 31 File: USPT Feb 26, 2002

DOCUMENT-IDENTIFIER: US 6350856 B1
TITLE: Cytokine suppressive anti-inflammatory drug binding protein

Detailed Description Text (62):
It is expected that p38beta2, like other MAP kinase, will be activated by a MAP kinase kinase, hence the recombinant protein would allow the establishment of a second assay which measures the ability of p38beta 2 to be phosphorylated by putative MAP kinase kinases. In this case fractions from stimulated cell lysates (eg THP.1 cells stimulated with LPS) are incubated with p38beta2 in the presence of .gamma.-.sup.32 P-ATP, and the incorporation of .sup.32 P-label into p38beta2 measured by separation and counting. Also, tyrosine phsophorylation of p38beta2 could be detected by immunoprecipitation or immunoblot with commercially available anti-phosphotyrosine antibodies.

☐ 18. Document ID: US 6319955 B1

L5: Entry 18 of 31 File: USPT Nov 20, 2001

DOCUMENT-IDENTIFIER: US 6319955 B1
**** See image for Certificate of Correction ****
TITLE: Use of MEK1 inhibitors as protective agents against damage due to ischemia

Drawing Description Text (6):
FIG. 5 shows that MEK inhibition blocks cell death induced by glutamate toxicity. (A) Extracellular glutamate (5 mM) induces death in HT22 cells by incubation for 10 hours. Cell viability was determined by the MTT assay; percent cell survival is presented as mean.+-.SEM (n=4). (B,C) Effects of U0126 (Promega), PD98059 (New England Biolabs) and SB203580 (Tocris) on injury in HT22 cells by incubating with glutamate (5 mM) for 9 hr (B) or 24 hr (C) was assessed by MTT assay 24 hr after addition of glutamate. Percent cell survival is presented as mean.+-.SEM (n=4). Pretreatment with SB203580 (50 .mu.M), an inhibitor of p38 MAP kinase and SAPKs/JNKs at this concentration, does not protect HT22 cells. PD98059 (50 .mu.M) and U0126 (10 .mu.M) MEK1-specific inhibitors attenuated cell injury (B, C). (D) Dose-dependent protection with U0126 in HT22 cells treated with glutamate (5 mM) for 24 hours (filled diamonds). U0126 is not toxic up to 10 .mu.M (open squares). (E) U0126 inhibits the phosphorylation of ERK1/2 in HT22 cells treated with glutamate, however does not affect total ERK1/2 protein levels. Immunoblotting was done with an antibody that specifically recognizes phosphorylated ERK1 and ERK2 (upper panel; New England Biolabs, dilution 1:1000) or with an antibody that recognizes ERK1/2 regardless of its phosphorylation state (lower panel; New England Biolabs, dilution 1:1000). (F) Effect of delayed application of U0126 on cell survival after glutamate toxicity. Ten .mu.M U0126 was added to HT22 cells at the indicated time after exposure to 5 mM glutamate, and cell survival was determined by MTT assay 24 hours after glutamate addition. Percent cell survival is presented as mean.+-.SEM (n=4). Application of U0126 was initiated up to 7 hours after glutamate completely inhibited cell death.

☐ 19. Document ID: US 6310060 B1

L5: Entry 19 of 31 File: USPT Oct 30, 2001

DOCUMENT-IDENTIFIER: US 6310060 B1
TITLE: 2-(4-bromo or 4-iodo phenylamino) benzoic acid derivatives and their use as MEK inhibitors

Detailed Description Text (61):
To determine the state of tyrosine phosphorylation of cellular MAP kinase, cells were lysed, endogenous MAP kinase was immunoprecipitated with a specific antibody, and the resulting immunoprecipitate analyzed for the presence of phosphotyrosine as follows: confluent cells were serum-deprived overnight and treated with compounds and growth factors as described above. Cells were then scraped and pelleted at 13,000.times.g for 2 minutes. The resulting cell pellet was resuspended and dissolved in 100 .mu.L of 1% SDS containing 1 mM NaVO.sub.4. Following alternate boiling and vortexing to denature cellular protein, 900 .mu.L RIPA buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% deoxycholate, and 10 mM EDTA) w as added. To this mixture was added 60 .mu.L agarose beads coupled with rabbit immunoglobulin m and 60 .mu.L Pansorbin cells in order to clear the lysate of nonspecific binding proteins. This mixture was incubated at 4.degree. C. for 15 minutes then centrifuged at 13,000.times.g for 10 minutes. The resulting supersatant was transferred to fresh tubes and incubated with 10 .mu.L of a polyclonal antisera raised against a fragment of MAP kinase for a minimum of 1 hour at 4.degree. C. Seventy microliters of a slurry of agarose beads coupled with protein G and protein A was added and the incubation continued for an additional 30 minutes at 4.degree. C. The beads were pelleted by centrifugation at 13,000.times.g for 5 minutes and washed three times with 1 mL RIPA buffer. Laemmli sample buffer was added to the final bead pellet. This mixture was boiled for 5 minutes then resolved on a 10% acrylamide gel. Proteins on the gel were transferred to a nitrocellulose membrane and nonspecific binding sites on the membrane blocked by incubation with 1% ovalbumin and 1% bovine serum albumin in TBST (150 mM NaCl, 10 mM Tris (pH 7.4), and 0.05% Tween 20). The membrane was then incubated with a commercially available a ntibody directed against phosphotyrosine. Antibody bound on the membrane was detected by incubation with .sup.125 I-protein A, followed by autoradiography.

☐ 20. Document ID: US 6300081 B1

L5: Entry 20 of 31 File: USPT Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6300081 B1
TITLE: Activated ras interaction assay

Brief Summary Text (11):
A primary target of activated Ras during growth factor stimulation is Raf, which is the first component of a protein kinase cascade that leads to activation of the MAP kinases Erk1 and Erk2 (Avruch et al., "Raf Meets Ras: Completing the Framework of a Signal Transduction Pathway," Trends Biochem. Sci., 19:279-83 (1994)). The phosphorylation of transcription factors by these MAP kinases results in the expression of immediate early response genes, such as c-fos, that are required for early G1 progression. Although these signalling events occur within minutes of growth factor stimulation, microinjection of neutralizing anti-Ras antibodies in late G1 phase blocks progression of fibroblasts into S phase (Mulcahy, et al., "Requirement for Ras Proto-oncogene Function During Serum-Stimulated Growth of NIH 3T3 Cells, Nature, 313:214-43 (1985)). Furthermore, studies using combinations of cell cycle inhibitors and anti-Ras microinjection clearly demonstrate multiple points of Ras requirement in early and late G1 phase (Dobrowolski et al., "Cellular Ras Activity Is Required for Passage Through Multiple Points of the G-0-G-1 Phase in BALB-c 3T3 Cells," Molecular and Cellular Biology, 14:5441-49 (1994). These findings, together with the observations that expression of oncogenic Ras increases cyclin D1 levels and shortens G1 phase (Liu, et al., "Ras Transformation Results in an Elevated Level of Cyclin D1 and Acceleration of G1 Progression in NIH 3T3 Cells," Mol. Cell Biol., 15:3654-63 (1995); Winston et al., "Regulation of the Cell Cycle Machinery by Oncogenic Ras," Oncogene, 12:127-34 (1996)) and that Ras and cyclin D1 cooperate in cellular transformation assays (Hinds et al., "Function of a Human Cyclin Gene as an Oncogene," Proc. Natl. Sci. USA, 91:709-13 (1994); Lovec et al., "Oncogenic Activity Cyclin D1 Revealed Through Cooperation with Ha-ras; Link Between Cell Cycle Control and Malignant Transformation," Oncocene, 9:323-26 (1994)) point to an important role for Ras in regulating progression from G1 into S phase.

☐ 21. Document ID: US 6291240 B1

L5: Entry 21 of 31 File: USPT Sep 18, 2001

DOCUMENT-IDENTIFIER: US 6291240 B1
TITLE: Cells or tissues with increased protein factors and methods of making and using same

Detailed Description Text (82):
Determination of mitogen-activated protein kinase (MAP) activation in fibroblast cells exposed to various stress/environmental stimuli was carried out as follows: ERK (Extracellular Receptor-activated Kinase), SAPK/JNK (Stress-Activated Protein Kinase/Jun Kinase) and p38 were estimated in 1% triton extracts of fibroblasts that had been exposed to various environmental stimuli using commercial kits for MAP kinase activity from New England Biolabs, Beverly, Mass. The MAP kinase was immunoprecipitated using a commercially available anti-MAP kinase antibody (New England Biolabs) and the precipitate was incubated with a specific artificial fusion protein substrate: Elk-1 for ERK, c-Jun for SAPK/JNK, and ATF-2 for p38 and ATP (New England Biolabs). The products were identified on Western blots using antibodies specific for the phosphorylated form of the substrate (New England Biolabs) and visualized by enzymatic chemiluminescence using the commercially available ECL kit Phototope (New England Biolabs).

☐ 22. Document ID: US 6288089 B1

L5: Entry 22 of 31 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6288089 B1
TITLE: Use of kinase inhibitors for treating neurodegenerative diseases

Detailed Description Text (170):
In this Example, experiments to observe p38 MAP kinase activity are described. Cultured embryonic rat mesencephalon tissue are cultured as described in Example 1. After washing with ice-cold HBSS, the cells are solubilized in 400 .mu.l of ice-cold immunoprecipitation buffer containing 10 mM Tris, pH 7.4, 1% Triton X-100, 0.5% nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium orthovanadate, and 0.2 mM phenylmethylsulfonyl fluoride. The cell lysates are centrifuged to remove insoluble material, and 200 .mu.g of the supernatant protein (400 .mu.L, total volume) are incubated with 1 .mu.g of anti-p38 antibodies for 1 hour at 4.degree. C. followed by incubation with 30 .mu.L of Protein G Plus/Protein Agarose for an additional hour. The immunocomplexes are pelleted and washed twice in immunoprecipitation buffer and then once in kinase wash buffer (50 mM .beta.-glycerophosphate, 1 mM EGTA, 20 mM MgCl.sub.2, 100 .mu.M sodium orthovanadate). The protein kinase assay is initiated by the addition of 20 .mu.L of 2.times. reaction buffer (50 mM .beta.-glycerol phosphate, 1 mM EGTA, 20 mM MgCl.sub.2, 100 .mu.M sodium orthovanadate, 0.1 mg/ml ATF-2 (N-terminal half), 50 .mu.g/ml IP20, a peptide inhibitor of cAMP dependent protein kinase, 200 .mu.M ATP, and 0.9 mCi/ml [.sup.32 P]ATP) to 20 .mu.L of immune complex. The reaction is allowed to proceed for 10 min at 30.degree. C. and then terminated by the addition of 2.times.Laemmli sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis using 12% acrylamide gels. After electrophoresis, the gels are dried and subjected to phosphoimaging (BioRad GS 100).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 23. Document ID: US 6258579 B1

L5: Entry 23 of 31 File: USPT Jul 10, 2001

DOCUMENT-IDENTIFIER: US 6258579 B1
TITLE: Stimulus-inducible protein kinase complex and methods of use therefor

Detailed Description Text (55):
NF.kappa.B activation is known to occur under conditions that also stimulate MAP kinase pathways (Lee et al., Cell 88:213-22, 1997; Hirano, et al., J. Biol. Chem. 271:13234-38, 1996). Accordingly, further experiments were performed to detect proteins associated with MAP kinase and phosphatase cascades at various stages of purification of the IKK signalsome. In addition to RelA and I.kappa.B.beta., MEKK-1 and two tyrosine-phosphorylated proteins of .about.55 and .about.40 kDa copurified with I.kappa.B kinase activity (FIG. 1C). Antibodies to Rel A and I.kappa.B.beta. were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.), and antibodies to MEKK-1 were obtained from Upstate Biotechnology (Lake Placid, N.Y.). Other signaling components, including PKC.zeta., PP1 and PP2A, were detected in the same fractions as the I.kappa.B kinase in early chromatographic steps but did not copurify at later chromatographic steps (data not shown). Most interestingly, an unidentified protein of .about.50 kDa, detected by its crossreaction with an antibody to MKP-1, copurified with I.kappa.B kinase through several purification steps (FIG. 1C). This protein is unlikely to be MKP-1 itself, since the molecular weight of authentic MKP-1 is 38 kDa.

☐ 24. Document ID: US 6251943 B1

L5: Entry 24 of 31 File: USPT Jun 26, 2001

DOCUMENT-IDENTIFIER: US 6251943 B1
TITLE: Method of treating or preventing septic shock by administering a MEK inhibitor

Detailed Description Text (195):
To determine the state of tyrosine phosphorylation of cellular MAP kinase, cells were lysed, endogenous MAP kinase was immunoprecipitated with a specific antibody, and the resulting inmmunoprecipitate analyzed for the presence of phosphotyrosine as follows: confluent cells were serum-deprived overnight and treated with compounds and growth factors as described above. Cells were then scraped and pelleted at 13,000.times.g for 2 minutes. The resulting cell pellet was resuspended and dissolved in 100 .mu.L of 1% SDS containing 1 mM NaVO.sub.4. Following alternate boiling and vortexing to denature cellular protein, 900 .mu.L RIPA buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% deoxycholate, and 10 mM EDTA) was added. To this mixture was added 60 .mu.L agarose beads coupled with rabbit immunoglobulin G and 60 .mu.L Pansorbin cells in order to clear the lysate of nonspecific binding proteins. This mixture was incubated at 4.degree. C. for 15 minutes then centrifuged at 13,000.times.g for 10 minutes. The resulting supernatant was transferred to fresh tubes and incubated with 10 .mu.L of a polyclonal antisera raised against a fragment of MAP kinase for a minimum of 1 hour at 4.degree. C. Seventy microliters of a slurry of agarose beads coupled with protein G and protein A was added and the incubation continued for an additional 30 minutes at 4.degree. C. The beads were pelleted by centrifugation at 13,000.times.g for 5 minutes and washed three times with 1 mL RIPA buffer. Laemmli sample buffer was added to the final bead pellet. This mixture was boiled for 5 minutes then resolved on a 10% acrylamide gel. Proteins on the gel were transferred to a nitrocellulose membrane and nonspecific binding sites on the membrane blocked by incubation with 1% ovalbumin and 1% bovine serum albumin in TBST (150 mM NaCl, 10 mM Tris (pH 7.4), and 0.05% Tween 20). The membrane was then incubated with a commercially available antibody directed against phosphotyrosine. Antibody bound on the membrane was

detected by incubation with .sup.125 I-protein A, followed by autoradiography.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 25. Document ID: US 6214795 B1

L5: Entry 25 of 31 File: USPT Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214795 B1
TITLE: Peptide compounds useful for modulating FGF receptor activity

Detailed Description Text (60):
The effect of peptide compounds on the functional activity of an FGF receptor can be evaluated in one or both of the functional assays described in this example. The first assay is a signal transduction assay, exploiting the fact that bFGF binding to FGFR initiates a phosphorylation cascade that includes the phosphorylation of MAP kinase (MAP-K). Accordingly, the ability of a test compound to modulate bFGF-induced phosphorylation of MAP-K is examined. NIH 3T3 cells are synchronized to quiescence by growing in medium containing 0.5% fetal bovine serum (FBS) for 2 days. The cells are then shifted into fresh 0.5% FBS-containing medium for 2 hours to reduce the basal level of MAP-K phosphorylation before the experiment. Test peptide compounds are dissolved in fresh dimethyl sulfoxide (DMSO) to 100 mg/ml and series dilutions are made in DMSO. Peptides at various dilutions are added to medium containing bFGF (1 or 10 .mu.M). Phosphorylation of MAP-K in the 3T3 cells is initiated by incubating the cells with the bFGF-containing medium in the presence or absence of test peptide for 15 minutes at 37.degree. C. The phosphorylation is stopped by washing with cells with PBS and lysing the cells with sodium dodecyl sulfide (SDS)-containing buffer. Cell lysates are separated on 12% SDS polyacrylamide gels and the proteins are transferred onto PVDF membranes. Membrane-bound cellular proteins are probed with a rabbit anti-phosphoMAP-K antibodies, followed by a goat anti-rabbit secondary antibody, labeled with horse radish peroxidase. The blots are then detected by the enhanced chemiluminescence (ECL) method.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 26. Document ID: US 6166289 A

L5: Entry 26 of 31 File: USPT Dec 26, 2000

DOCUMENT-IDENTIFIER: US 6166289 A
TITLE: IRAK modified transgenic animals

Detailed Description Text (60):
In vitro kinase assay. After stimulation, cells were lysed in NP40 lysis buffer (20 mM Hepes pH 7.5, 150 mM NaCl, 1% Nonidet P 40, 1 mM Na.sub.3 VO.sub.4) containing EDTA-free complete protease inhibitor cocktail (Boehringer Mannheim Corp., Indianapolis, Ind.), centrifuged at 16,000.times.g for 10 minutes, and precleared twice with 50 1 of GammaBind G Sepharose slurry (Pharmacia Biotech, Inc., Piscataway, N.J.). MAP kinases were immunoprecipitated with 50 1 GammaBind G Sepharose slurry and 2 .mu.g polyclonal rabbit antibody, specific for the 20 C-terminal residues of p38 .alpha. or the 17 C-terminal residues of JNK1 (Santa Cruz Biotechnology, Santa Cruz, Calif.). Immunoprecipitates were washed twice with NP40 lysis buffer and twice with kinase reaction buffer (25 mM HEPES pH 7.5, 10 mM MgCl.sub.2, 10 mM MnCl.sub.2, 20 mM .beta.-glycerophosphate, 1.times. EDTA-free complete protease inhibitor cocktail). Kinase reactions were performed for 20 minutes at 30.degree. C. in kinase reaction buffer containing 50 .mu.M ATP and 5 .mu.Ci .gamma.-.sup.32 P-ATP (3000 Ci/mmol, Amersham Corp., Arlington Heights, Ill.) with 0.5 .mu.g GST-MAPKAPK2 (Upstate Biotechnology, Lake Placid, N.Y.) as p38 substrate or 2 .mu.g GST-c-JUN (BioMol, King of Prussia, Pa.) as JNK-1 substrate. Samples were boiled in SDS sample buffer, electrophoresed in 10% Tris-Glycine polyacrylamide gels and transferred to PVDF membranes (Novex, San Diego, Calif.). Bands were quantified on a Storm 840 PhosphorImager System (Molecular Dynamics Inc., Sunnyvale, Calif.). Membranes were stained for p38 and JNK1 protein using the p38 and JNK1 antibodies described above and the Vistra ECF Western blotting system (Amersham).

☐ 27. Document ID: US 6136596 A

L5: Entry 27 of 31 File: USPT Oct 24, 2000

DOCUMENT-IDENTIFIER: US 6136596 A
TITLE: Cytokine-, stress-, and oncoprotein-activated human protein kinase kinases

Detailed Description Text (145):
To examine the specificity of MKK7 in vivo, cotransfection assays were performed. CHO cells were maintained in Dulbecco's modified Eagle's medium supplemented with fetal calf serum (5%; Gibco-BRL). The cells were transfected with the lipofectamine reagent according to the manufacturer's recommendations (Gibco-BRL) Derijard (1994) supra). Cells were co-transfected with vectors encoding epitope-tagged JNK1 together with an empty expression vector (control) or an expression vector encoding MKK4 or MKK7. The epitope tag was derived from the hemagglutinin protein (HA) of the influenza virus. JNK1 was isolated by immunoprecipitation of cell lysates. The cells were solubilized with lysis buffer (20 mM Tris (pH 7.4), 1% TRITON X-1000, 10% glycerol, 137 mM NaCl, 2 mM EDTA, 25 mM .gamma.-glycerophosphate, 1 mM Na orthovanadate, 2 mM pyrophosphate, 1 mM PMSF, 10 .mu.g/ml leupeptin) and centrifuged at 100,000.times.g for 15 minutes at 4.degree. C. The epitope-tagged protein kinases were immunoprecipitated by incubation for 3 hours at 4.degree. C. with an anti-HA monoclonal antibody bound to protein-G Sepharose (Pharmacia-LKB Biotechnology Inc.). The immunoprecipitates were washed three times with lysis buffer (Gupta et al. (1995) Science 267:389-393). Protein kinase activity was measured in the immunecomplex with [.gamma.-.sup.32 P]ATP and

c-Jun as substrates. The product of the phosphorylation reaction was visualized after SDS-PAGE by autoradiography. The ERK2 and p38 MAP kinases were not activated by co-expressed MKK7. Control experiments demonstrated that the ERK2 and p38 MAP kinases were activated by their respective cognate MAP kinase kinases, MKK1 and MKK6. In contrast, MKK7 did activate JNK1. Interestingly, the activation of JNK1 by co-expressed MKK7 was greater than that caused by the previously described JNK activator MKK4. Together, these data establish that MKK7 can function as a specific activator of JNK in cultured cells.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 28. Document ID: US 6001580 A

L5: Entry 28 of 31 File: USPT Dec 14, 1999

DOCUMENT-IDENTIFIER: US 6001580 A
TITLE: Method for assaying ERK2 map kinase

Detailed Description Text (274):
The monoclonal antibody HE113 specific for human MAP kinase ERK1, prepared in Example 2, and the polyclonal antibody (anti-peptide-3 antibody) specifically binding to MAP kinase ERK1, prepared in Example 9, were respectively diluted to 20 .mu.g/ml with 10 mM NaHCO.sub.3 buffer (pH 8.0) and added to a 96-well EIA plate (Corning, No. 430480), 100 .mu.l per well. Each assay plate was then allowed to sit at 4.degree. C. overnight so as to let the antibody immobilized to the solid phase. After the wells were rinsed twice with PBS (8.1 mM disodium phosphate, 1.5 mM potassium phosphate, 27 mM potassium chloride, 137 mM NaCl, pH 7.2), 300 .mu.l/well of PBS containing 25% of Block Ace (Snow Brand Milk Products Co.) was added and the plate was stored cold until needed.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 29. Document ID: US 5731343 A

L5: Entry 29 of 31 File: USPT Mar 24, 1998

DOCUMENT-IDENTIFIER: US 5731343 A
TITLE: Method of use of radicicol for treatment of immunopathological disorders

Detailed Description Text (65):
The time course of protein tyrosine phosphorylation in LPS-stimulated macrophages showed that the maximum

phosphorylation occurred within 1 hour whether cells were treated with radicicol or not (FIG. 2A). Both MAPK- 1 (42 KD) and MAPK-2 (44 KD) were detected in cell lysates derived from the time course study as determined by Western blot analysis using polyclonal anti-rat MAP kinase antibodies recognizing both MAPK-1 and MAPK-2 (FIG. 2B). However, these MAP kinases did not appear to be tyrosine phosphorylated significantly at the incubation conditions used in our studies (FIG. 2A). Tyrosine phosphorylated MAPKs were not detected by the anti-phosphotyrosine immunoblot procedure described herein. When 2% BSA, instead of 3% dry milk was utilized in the blocking buffer, tyrosine phosphorylated MAPKs were detectable as shown in FIG. 2D. FIG. 2D shows the inhibition of tyrosine phosphorylation of mitogen activated protein kinases by radicicol in macrophage cell line (RAW 264.7, ATCC). Cells were pretreated with the indicated concentrations of radicicol for four hours, and then stimulated with LPS (1 .mu.g/ml). Solubilized proteins were analyzed by antiphosphotyrosine immunoblotting as described in FIG. 2A. Tyrosine phosphorylated MAPK bands were identified using polyclonal antibodies recognizing both MAPK-1 and MAPK-2, and polyclonal antibodies for p38. p38 is recently cloned isoform of MAPK (Han, et al., Science, 265:808, 1994).

☐ 30. Document ID: US 5723300 A

L5: Entry 30 of 31 File: USPT Mar 3, 1998

DOCUMENT-IDENTIFIER: US 5723300 A
TITLE: Nuclear localized transcription factor kinase and diagnostic assays related thereto

Detailed Description Text (12):
Consistent with a large body of work on "switch kinases" and nuclear phosphorylation (Ahn and Krebs, 1990; Pelech et al., 1987; 1990) that has failed to detect phosphotyrosine phosphorylation in the nucleus, the 90 kD species contained no phosphotyrosine, and phosphorylated its myelin basic protein substrate only on serine. Myelin basic protein is used frequently as an in vitro substrate for MAP kinases, and its suitability here suggested that the 90 kD kinase might share some structural or functional similarities with the members of the MAP kinase family or with the RSK family, which is immunologically related to the MAP kinases. However, the 90 kD kinase did not phosphorylate a range of substrates, including peptide substrates for S6 kinase, MAP kinase, casein kinase II, glycogen synthase kinase III, protein kinase C, calcium-calmodulin-dependent protein kinase, or Raytide (a general substrate for tyrosine kinases). It did phosphorylate peptide substrates for smooth muscle myosin light chain kinase and for protein kinase A and was active in the absence of cofactors. These substrate peptides have at least two hydrophobic, basic amino acids that are positioned two residues toward the N-terminus from a phosphorylatable serine (Kemp and Pearson, 1990). Amino acid sequence analysis of the phosphorylation site and site-directed mutagenesis will provide more information about the substrate requirements of the kinase. The nuclear localization of the enzyme and its lack of immunoreactivity with rabbit polyclonal antibodies to protein kinase C, RSK or MAP kinases indicated that the 90 kD kinase was unlike other previously characterized kinases.

Detailed Description Text (22):
Dextran sulfate (average molecular weight 500,000), insulin, glucagon, acidic fibroblast growth factor,

reactive green-19 and Cibacron Blue 3GA (Type 3000) agarose were from Sigma. Sodium orthovanadate, Tris, trichloroacetic acid, MgCl.sub.2 and NaOH were from Fisher. Disodium ATP and NP-40 were from Pharmacia LKB. Guanidine hydrochloride was from Fluka. Phytohemagglutinin-M, phorbol 12-myristate 13-acetate, calcium ionophore A23187, human interleukin-2, (7.beta.-deacetyl-7.beta.-[-N methylpiperazino]-buytyrl)-forskolin dihydrochloride and rat brain protein kinase C were from Calbiochem. Wheat germ extract and rabbit reticulocyte extract systems for in vitro translation were from Promega. Raytide and recombinant p43.sup.v-abl were from Oncognene science (Uniondale, N.Y.). In vitro phosphorylation substrates were from Peninsula Laboratories (Belmont, Calif.). [.alpha.- and .gamma.-.sup.32 P]ATP were from New England Nuclear. Nitrocellulose membranes were from Micron Separations (Westboro, Mass.) and polyvinyl difluoride membranes (PVDF) were from Millipore. Recombinant protein A-agarose was from Repligen (Cambridge, Mass.). Phosphocellulose resin (P-11) was from Whatman. Polyacrylamide monomer, bisacrylamide and ammonium persulfate were from National Diagnostics (Manville, N.J.). Cell culture media, calf serum, monoclonal antibody Mab 1.9 against rat brain protein kinase C and TEMED were from GIBCO-BRL. Fetal bovine serum for Jurkat cell culture was from Whittaker Bioproducts (Walkersville, Md.). Cell lines (HeLa, ATCC CCL 2; A431, ATCC CRL 1555; Jurkat, ATCC TIB 152; HUT78, ATCC TIB 161; Mvl Leu, ATCC CCL64; CHO DUKX B1, ATCC CRL 9010; CV-1, ATCC CCL70; and COS-7, ATCC CRL 1651) were obtained from ATCC and cultured as advised. Jurkat clone E6-1 (Weiss et al., 1984) and HUT78 (Gazdar et al., 1980) were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. 3T3-L1 fibroblasts (ATCC CL 173) were the kind gift of Michael P. Czech, University of Massachusetts Medical Center, and were differentiated according to Rubin et al. (1978). An Epstein-Barr virus-transformed human B-cell line (32D clone 13) (FitzGerald et al., 1991) was obtained from Joel Greenberger, University of Massachusetts Medical Center. Human peripheral blood lymphocytes from normal, healthy volunteers, were the kind gift of John Sullivan University of Massachusetts Medical Center. Rabbit polyclonal antisera to purified GST fusion proteins were prepared by Berkeley Antibody Company (Richmond, Calif.). Platelet-derived growth factor was the kind gift of Roger J. Davis, University of Massachusetts Medical Center. Rabbit anti-rat MAP kinase (erk-1 C-terminal 333-367 amino acids) polyclonal antibody was from Upstate Biotechnology (Lake Placid, N.Y.) and anti-RSK rabbit antibody was the kind gift of John Blenis, Harvard Medical School. HeLa nuclear extract was prepared with 1 mM sodium vanadate and 50 mM .beta.-glycerophosphate after the method of Dignam et al. (1983). All other reagents were from Sigma.

☐ 31. Document ID: US 5525625 A

L5: Entry 31 of 31 File: USPT Jun 11, 1996

DOCUMENT-IDENTIFIER: US 5525625 A
TITLE: 2-(2-Amino-3-methoxyphenyl)-4-oxo-4H-[1]benzopyran for treating proliferative disorders

Detailed Description Text (26):
To determine the state of tyrosine phosphorylation of cellular MAP kinase, cells were lysed, endogenous MAP kinase was immunoprecipitated with a specific antibody, and the resulting immunoprecipitate analyzed for the presence of phosphotyrosine as follows: confluent cells were serum-deprived overnight and treated with

compounds and growth factors as described above. Cells were then scraped and pelleted at 13,000.times.g for 2 minutes. The resulting cell pellet was resuspended and dissolved in 100 .mu.L of 1% SDS containing 1 mM NaVO.sub.4. Following alternate boiling and vortexing to denature cellular protein, 900 .mu.L RIPA buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% deoxycholate, and 10 mM EDTA) was added. To this mixture was added 60 .mu.L agarose beads coupled with rabbit immunoglobulin G and 60 .mu.L Pansorbin cells in order to clear the lysate of nonspecific binding proteins. This mixture was incubated at 4.degree. C. for 15 minutes then centrifuged at 13,000.times.g for 10 minutes. The resulting supernatant was transferred to fresh tubes and incubated with 10 .mu.L of a polyclonal antisera raised against a fragment of MAP kinase for a minimum of 1 hour at 4.degree. C. Seventy microliters of a slurry of agarose beads coupled with protein G and protein A was added and the incubation continued for an additional 30 minutes at 4.degree. C. The beads were pelleted by centrifugation at 13,000.times.g for 5 minutes and washed 3 times with 1 mL RIPA buffer. Laemmli sample buffer was added to the final bead pellet. This mixture was boiled for 5 minutes then resolved on a 10% acrylamide gel. Proteins on the gel were transferred to a nitrocellulose membrane and nonspecific binding sites on the membrane blocked by incubation with 1% ovalbumin and 1% bovine serum albumin in TBST (150 mM NaCl, 10 mM Tris (pH 7.4), and 0.05% Tween 20). The membrane was then incubated with a commercially available antibody directed against phosphotyrosine. Antibody bound on the membrane was detected by incubation with 125I-protein A, followed by autoradiography.

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